



# Biodegradation of dispersed oil in natural seawaters from Western Greenland and a Norwegian fjord

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## Abstract

With increasing oil exploration, production and transportation in the Arctic, predictions of the fate of spilled oil become important, including oil compound biodegradation. The use of chemical dispersants may result in increased biodegradation due to the generation of dispersions consisting of small oil droplets, but only few studies have focused on biodegradation of chemically dispersed oil in Arctic seawater. In this study we compared oil biotransformation in Arctic and temperate seawaters collected from Western Greenland (Disko Bay, surface and from 80 m depth) and a Norwegian fjord (Trondheimsfjord, surface). A naphthenic oil, premixed with a dispersant, was dispersed in the seawaters from the different sources, and the dispersions incubated in low concentrations in a carousel system at 4–5 °C for up to 64 days. Targeted oil compounds (*n*-alkanes, BTEX, naphthalenes and PAHs) were biotransformed in both Arctic and temperate seawaters, although the degradation was faster in the temperate seawater. In the Arctic seawater, transformation was faster in the surface than in the subsurface seawater. Calculations of biotransformation rates and half-lives of oil compound groups representing 70–80% of fresh oils also showed significantly faster depletion in the temperate than the Arctic seawater. Microbial analyses revealed differences between the bacterial communities in the seawater sources during oil biodegradation. The results emphasized, that oil compounds are biodegraded in Arctic seawater, but degradation potential and rates may vary between seawaters from different sources.

**Keywords** Oil · Dispersants · Dispersibility · Biodegradation · Seawater

## Introduction

The Arctic regions represent areas of interest for oil exploration and production. According to an assessment made by the U.S. Geological Survey, the estimated occurrence of undiscovered oil and gas north of the Arctic Circle may be as much as 90 billion barrels of oil and 1669 trillion

cubic feet of natural gas, most of it in offshore areas (Bird et al. 2008). The Greenland basin alone is estimated to contain 17 billion barrels of oil and 138.000 billion cubic feet of natural gas, and several offshore exploration licences have been granted west of Greenland (Olsen and Hansen 2014). Reduced ice coverage in the Arctic may also result in increased transport activities. Despite strict governmental regulations, accidental releases of oil from exploration, production and transport may occur and cause impacts on the local marine environments in these vulnerable areas. Oil spilled to the marine environment undergoes a number of weathering processes like evaporation, water-in-oil (w/o) emulsification, dispersion, dissolution of small and charged compounds, and photo-oxidation (NRC 2003). Biodegradation is also an important weathering process and may result in complete mineralization of hydrocarbons. However, the rates and extents of the degradation depend strongly on a variety of factors involving oil characteristics and environmental conditions. Oil compounds may be degraded by a variety

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of marine microbes, including bacteria, fungi, and algae (Prince 2005). While hydrocarbon-degrading bacteria in temperate seawater usually include members of the classes Alphaproteobacteria and Gammaproteobacteria, oil-polluted Arctic seawater and marine ice often become enriched by Gammaproteobacteria (Bowman and McCuaig 2003; Yakimov et al. 2004; Deppe et al. 2005; Gerdes et al. 2005; Brakstad et al. 2008; Bagi et al. 2014; McFarlin et al. 2014a; Garneau et al. 2016). In particular, the *Exxon Valdez* grounding in Prince William Sound in 1989 drew public attention to oil biodegradation of oil discharged in the Arctic, and bioremediation was used as a secondary treatment of stranded oil in the Arctic (Bragg et al. 1994).

Oil biodegradation is mainly associated with the dissolved and dispersed fractions of the oil. Small oil-droplet dispersions generate large surfaces which hydrocarbonoclastic bacteria may attach to, and several studies have shown that the use of oil spill dispersants may stimulate oil biodegradation (Siron et al. 1995; Venosa and Holder 2007; Bælum et al. 2012; Prince et al. 2013; Brakstad et al. 2014; McFarlin et al. 2014b; Techtmann et al. 2017). By encouraging the formation of small oil droplets, dispersants increase the surface-to-volume ratio of the oil and enhance oil biodegradation by creating larger oil surfaces for microbial attachment, but also dissolution of soluble compounds may be increased compared to oil emulsions. Efficient use of oil spill dispersants typically results in oil-droplet sizes of 10–20  $\mu\text{m}$  diameter at breaking wave conditions (Brakstad et al. 2014). Some laboratory studies have also indicated negligible or uncertain effects of dispersants on oil compound biodegradation rates (Swannell and Daniel 1999; Lindstrom and Braddock 2002; Macnaughton et al. 2003), or even suggested that dispersants may suppress the activity of oil-degrading microbes (Kleindienst et al. 2015). However, these studies have often either been performed with very high oil concentrations, or dispersed oil has not been used. Once the oil is chemically dispersed, rapid dilution will occur in the water column. Environmental concentrations of dispersed oil over the period of biodegradation is expected to be below  $1 \text{ mg L}^{-1}$  (Lee et al. 2013). It has also been argued that the use of dispersants may be of advantage in the Arctic, since the chemicals can be applied by plane and are therefore less logistically challenging than mechanical treatment (Lewis and Prince 2018).

Cold seawater affects several physical processes. Lower temperatures will increase the oil viscosity. Evaporation will be also reduced, resulting in temporary higher concentrations of volatile compounds in the seawater (e.g. BTEX). For wax-rich oils with high pour points, evaporation, dissolution, and dispersion may be reduced in cold seawater. Precipitated wax may build a matrix which limits the internal mixing of the oil and acts as a diffusion barrier between the oil and the water.

In order to predict the fate of spilled oil in the marine environment, three-dimensional and dynamic oil spill models like OSCAR have been developed (Reed et al. 1995, 2000). These models include descriptions of the physical environment and the physical–chemical fate processes of the spilled oil. The strength of such multi-component models is that the contribution of the various fate processes can be estimated by mass balance calculations (Reed et al. 1995). Biodegradation has been included in the OSCAR model as part of the fate predictions of spilled oil, using biotransformation rates of 25 oil compound groups expecting to represent approximately 70–80% of the oil (Reed et al. 2000; Pasquini and Bueno 2007). The biodegradation rates have been obtained from laboratory studies conducted at  $13 \text{ }^\circ\text{C}$  in natural temperate seawater (Brakstad and Faksness 2000). Temperature calibration of the biotransformation rates are currently performed by a  $Q_{10}$  approach ( $Q_{10}=2$ ) (Bagi et al. 2013).

While numerous oil/hydrocarbon biodegradation studies have been performed with oil in seawater from temperate regions, fewer studies have been performed in cold Arctic or Antarctic seawater (e.g. Delille et al. 1998a, b, 2009; Bagi et al. 2014; McFarlin et al. 2014b; Kristensen et al. 2015; Scheibye et al. 2017). In this study, we compared biodegradation of small oil-droplet dispersions at low temperature, using natural non-amended seawater from Western Greenland (Disko Bay) and temperate seawater from a Norwegian fjord acclimated to low temperature. The objective of the study was to investigate if biodegradation of oil compounds in dispersed oil differed in the two seawater sources at similar experimental conditions. By this study, we aimed to obtain data to evaluate if biodegradation as input parameter in oil spill models like OSCAR should be generated as site-specific data for predictions of the fate of oil spills in Arctic seawater.

## Materials and methods

### Seawater sampling and measurements

Arctic seawater from Western Greenland was collected on 11. September 2016 in Disko Bay (DB), close to the Arctic Station in Qeqertarsuaq, a small town on Disko Island ( $69^\circ 15' \text{N}$ ,  $53^\circ 34' \text{W}$ ), see map in Supplementary Material (Online Resource 1). Vertical profiles of temperatures, salinities and densities were measured by a CTD (see Supplementary Material, Online Resource 2). Seawater was collected in a Niskin bottle sampler (10 L volume). Volumes of 60 L were collected from near the surface (2.5 m depth) and 40 L from 80 m depth. Seawater temperatures were  $5 \text{ }^\circ\text{C}$  in the surface and  $2 \text{ }^\circ\text{C}$  in the sub-surface seawater. The seawater samples were transported in 19 L (5 gallon)

Teflon (Polytetrafluoreten) bags (Welch Fluorocarbon, Inc., Dover, NH, USA). The bags were closed with double sets of pull-ties and placed in transport coolers (PeliCase coolers, Pelican Products, Inc., Torrance, CA, USA). Free space in the PeliCase coolers were packed with frozen wet towels, and temperature loggers were placed in each cooler, with temperatures logged every 30 min. The seawater samples were transported by boat to the mainland, and by plane from Ilulissat to SINTEF's laboratories in Trondheim, Norway. The seawater arrived at SINTEF on 14. September 2016 and was placed at 4 °C until start of the experiment. The seawater transport in the coolers secured low temperatures, with a median temperature of 4–5 °C during the first 28 h, followed by a slow median increase to 7 °C before the coolers reached our laboratories after 52 h of transport. Only one cooler reached a peak of 9 °C the last hour before it was placed at 4–5 °C (Online Resource 3).

Temperate seawater was collected from the Trondheimsfjord (TRD; 63°26'N, 10°23'E), from the upper 0 to 0.5 m of the surface (60 L) in 10 L barrels (10. September 2016). Seawater temperature was 11° when sampled. The seawater was transported to the SINTEF laboratories and placed at 4 °C until start of the experiment.

Surface and subsurface seawater samples from DB (approximately 1 L) were filtered on-site for microbial community analyses (surface and subsurface) in triplicate samples through 0.22 µm Durapore membrane filters, and the filters placed in Eppendorf tubes. Filters with DB seawater were placed in Isopore boxes with frozen cooling elements, transported in the PeliCase coolers together with the seawater, and stored at – 20 °C after arrival at SINTEF.

### Biodegradation experiment

A biodegradation experiment was started on 19. September 2016. A fresh naphthenic oil (Troll C; pour point – 18 °C, density of 0.900 g cm<sup>-3</sup>) was mixed with the dispersant Corexit 9500A (Nalco Environmental Solutions LLC, Sugar Land, TX, USA) at a dispersant-to-oil ratio (DOR) of 1:100. An oil-droplet generator was used for preparing oil dispersions with defined droplet size distributions, as previously described (Nordtug et al. 2011). The system consists of three chambers with an inner diameter of 8 mm connected by nozzles with a diameter of 0.5 mm. Stock oil dispersions with defined oil-droplet size distributions (median diameter 20 µm) and concentrations (200 mg L<sup>-1</sup>) were generated by injecting oil via a capillary into a flow of non-amended filtered (1 µm exclusion limit) seawater (acclimated to 4–5 °C for 5 days) moving through the chambers of the droplet generator. Oil-droplet concentrations and size distribution were determined by Coulter Counter analyses (see below).

Flasks (2 L; Schott) were baked, washed, sterilized, and filled with corresponding seawater (acclimated to 4–5 °C

for 5 days) from the different water sources. Oil dispersion stock solutions were diluted in unfiltered seawater to nominal concentrations of 3 mg L<sup>-1</sup>, based on Coulter Counter analyses (see below). Flasks were completely filled with the respective seawater to avoid any headspace or air bubbles and capped (PBT screw caps and PTFE protected seal). The flasks were immediately mounted on a carousel system with slow continuous axial rotation (0.75 r.p.m), as previously described (Brakstad et al. 2015a). The dispersions were incubated on the carousel system at 4–5 °C for up to 64 days. This temperature corresponded to the surface seawater temperature in DB during water sampling (Supplementary Material, Online Resource 2). Seawater blanks containing only unfiltered seawater without oil were also included in the experiment. Flasks with dispersions in Arctic seawater from surfaced or subsurface (triplicate), and temperate seawater from surface (triplicate) were sampled for analyses after 0 (15 min on carousels), 7, 14, 21, 28, 42, and 64 days. Seawater blanks from all three localities were sampled similarly (one replicate of each).

### Particle analyses

Oil-droplet concentrations and size distributions in 20 mL samples (triplicate) were determined by Coulter Counter measurements (Beckman Multisizer 4; Beckman Coulter Inc., Brea, CA, USA) fitted with a 100 µm aperture for measurement of droplets size and concentrations within a diameter range of 2–60 µm. Filtered (0.22 µm) seawater was used as electrolyte. All droplet sizes reported here are expressed as median droplets diameter.

### Chemical analyses

Samples of oil dispersions and seawater blanks (1 L) were acidified (pH < 2) and solvent–solvent extracted with dichloromethane (DCM) for measurements of semivolatile organic compounds (SVOC). The flask glass walls and interior of lids were also rinsed with DCM after removal of dispersions to extract material attached to the glass walls. GC-FID analyses (Agilent 6890 N with 30 m DB1 column; Agilent Technologies) were used for quantification of total extractable material (TEM), representing mainly saturates (linear, branched and cyclic alkanes). GC-MS analyses (Agilent 6890 plus GC coupled with an Agilent 5973 MSD detector, operated in Selected Ion Monitoring [SIM] modus; Agilent Technologies) were used for analyses of targeted SVOC compounds, including nC<sub>10</sub>–nC<sub>36</sub> n-alkanes, decalines, phenols, 2- to 5-ring polyaromatic hydrocarbons (PAHs) and 17α(H),21β(H)-Hopane (30ab Hopane), as recently described (Brakstad et al. 2014). For analyses of volatile organic compounds (VOCs), dispersions or seawater blanks (40 ml) were acidified (pH < 2) and analysed for targeted

compounds (C<sub>5</sub>–C<sub>10</sub> alkanes and monoaromatic hydrocarbons) in a Purge & Trap unit (Teledyne Tekmar Atomx; Mason OH, USA) coupled to a GC–MS (Agilent 6890 N GC and an Agilent 5975B MSD detector; Agilent Technologies) (P&T GC–MS). The response values for individual target analytes were determined, with a signal-to-noise ratio of 10 as the lower detection limit, and a lower limit of detection (LOD) of 0.01 µg L<sup>-1</sup> was defined for all individual oil compounds analysed by GC–MS (VOC and SVOC), while a LOD for GC-FID analyses (TEM) was 0.1 µg L<sup>-1</sup>. VOC and SVOC compounds were grouped in 23 of the oil compound groups described for the OSCAR oil spill model (Reed et al. 2000; see Online Resource 4).

### Microbial counts

Tenfold dilutions in deionized water of oil dispersions or seawater samples were incubated in 4',6-diamidino-2-phenylindole (DAPI; 0.2 µg mL<sup>-1</sup>) for 10 min, and filtered through 0.2 µm black polycarbonate filters (Nucleopore, Costar, Cambridge, MA, USA). Total counts of prokaryotic cells were enumerated by oil immersion fluorescence microscopy at 1250 times magnification. Ten individual counts were performed and concentrations (cell mL<sup>-1</sup> sample) determined based on an ocular lattice system and dilution used for counting.

Most probable number (MPN) analyses of aerobic heterotrophic prokaryotes (HP) and oil-degrading prokaryotes (ODP) were performed in tenfold dilutions of sterile-filtered (0.22 µm) seawater. Each dilution (0.2 mL) was applied in triplicate to 1.8 mL Marine Broth 2216 (Difco Labs, Detroit, MI, USA) in 24-well tissue culture plates (Costar) for HP analyses, or to 1.8 mL while Bushnell-Haas broth (Difco) in 24-well tissue culture plates, supplemented with 30 g L<sup>-1</sup> NaCl and with 0.1% (vol/vol) fresh oil (Troll) as carbon source. The MPN-plates for HP were incubated at 4–5 °C for 7 days. The plates were then incubated at room temperature for 3 days to boost the growth in wells with dubious growth, before final analyses. Based on previous experience with slow growth at 4–5 °C, MPN-plates for analyses of ODP were incubated at room temperature for 14 days before recording. At the end of the test, actively respiring ODP cultures were detected by adding 100 µl fluorescein diacetate (0.1 mg mL<sup>-1</sup> final concentration) to each well with Bushnell-Haas media for 60 min before the results were recorded. Growth was recorded with respect to dilution, and final concentrations of viable HP and ODP determined from standard MPN tables.

### Microbial community analyses

Samples (500 mL) collected during biodegradation were filtered through 0.22 µm Durapore filters, and filters

placed in Eppendorf tubes and frozen (– 20 °C). DNA samples on filters with Arctic or temperate seawater, and from the biodegradation experiment, were extracted by FastDNA Spin kit for soil (MP Biomedicals), according to the manufacturer's instructions. DNA yields were quantified using Qubit 3.0 (Thermo Fisher Scientific, MA, USA) with dsDNA High Sensitivity kit (ThermoFisher Scientific, MA, USA). 16S rDNA amplicons were generated by PCR using primer combinations targeting the V3 and V4 hypervariable regions of bacterial 16S rRNA genes (Klindworth et al. 2013). Primers were tagged with Illumina adapter overhang and hot-start PCR performed (Eppendorf Mastercycler) to generate 16S rDNA amplicons. The generated amplicons were isolated using PCR clean-up spin columns (Qiagen; QIAquick Gel Extraction Kit), and thereafter used in index PCR with Illumina NEXTERA XT indices. Indexed 16S amplicons were isolated using PCR clean-up spin columns, verified and quantified (Qubit 3.0), and DNA indexed amplicons were diluted and pooled for generation of a library for sequencing. DNA library and Illumina positive control PhiX samples were thereafter denatured. DNA library pool and PhiX were then incubated at 96 °C for 2 min before running in an Illumina MiSeq sequencer according to Illumina's 16S rRNA sample preparation guideline.

16S rRNA gene sequences were analysed using QIIME pipeline (Caporaso et al. 2010) and R language package Phyloseq (McMurdie and Holmes 2013), and the reads were checked for chimera sequences against ARB Silva database. Operational taxonomic units (OTUs) were based on cut-off of 97% of sequence similarity, and taxonomy assignment was performed with RDP classifier (Wang et al. 2007). The raw reads of each OTU were converted to relative abundances and taxa were agglomerated on the desired taxonomic level between replicates of the identical sampling point for the purpose of OTU comparison across incubation days and treatments. Representation of the most abundant prokaryotes was obtained by selecting the taxa that contained at least 10% of abundance at any time point. Taxa with relative abundances less than 10% were assigned as "Other".

### Other analyses

Dissolved oxygen (DO) and water temperatures were determined by a DO meter (YSI, Inc., Yellow Springs, OH, USA).

Nutrient analyses of seawater included total Nitrogen (internal procedure), NO<sub>3</sub> + NO<sub>2</sub>-N (ISO 13395), NH<sub>4</sub>-N (ISO 11732), *o*-PO<sub>4</sub>-P and total Phosphorous (both ISO 15681-2), Iron (ISO 17294-2:2016), and total organic carbon (TOC) (EN 1484), all analysed by Eurofins Environment Testing Norway, Bergen, Norway.

## Data treatment and statistics

Non-linear regression analyses were performed by the option "plateau followed by one-phase decay" in GraphPad Prism vs. 6.0 (GraphPad Software Inc., La Jolla, CA, USA). The "plateau" represented the non-responsive lag-periods. Half-lives were determined from rate coefficients ( $t_{1/2} = 0.693/k_1$ ). We were unable to categorically exclude abiotic losses for volatile oil compounds analysed by P&T GC-MS, since sterilized controls were not included and these compounds could not be normalized against 30ab Hopane. Revised biotransformation half-lives ( $t_{1/2\text{-rev}}$ ) and rate coefficients ( $k_{1\text{-rev}}$ ) were determined, in which the influences of the non-responsive lag-periods also were accounted for:  $t_{1/2\text{-rev}} = \text{lag-period} + t_{1/2}$ , and  $k_{1\text{-rev}} = 0.693/t_{1/2\text{-rev}}$ . For comparison, biotransformation rate coefficients and half-lives were determined as standard first-order rates ("one-phase decay"; GraphPad Prism), without including any lag-periods in the calculations. The determination of rate coefficients ( $k_1$ ) and half-lives ( $t_{1/2}$ ) by standard first-order rates, and the determination of revised rate coefficients ( $k_{1\text{-rev}}$ ) and half-lives ( $t_{1/2\text{-rev}}$ ), are described for the OSCAR group PAH-1 in DB surface seawater in Online Resource 5).

$Q_{10}$  calculations of rate coefficients at 5 °C ( $k_{15}$ ) were made with rate coefficients from experimental data generated at 13 °C,  $k_{113}$  (Brakstad et al. 2018a), based the equation  $Q_{10} = 2$  (rate coefficients increased by a factor of 2 for temperature increase of 10 °C):  $k_{15} = k_{113} \times 10^{0.03(t_5 - t_{13})}$ , where 0.03 is a  $Q_{10}$  conversion factor, and  $t_5$  and  $t_{13}$  represent the temperatures of 5 and 13 °C, respectively (Bagi et al. 2013).

Column statistics were compared by Wilcoxon matched paired  $t$  test in GraphPad Prism vs. 6.

## Results

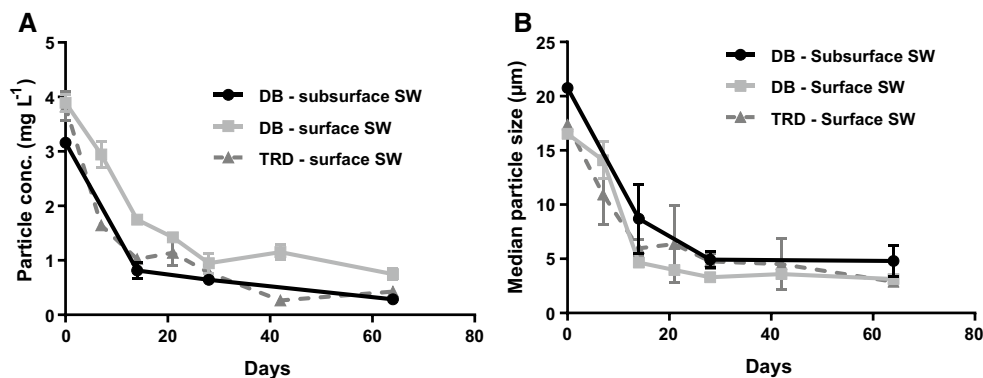
### Seawater sampling and transport

Vertical CTD monitoring of the water column at the sampling site in DB (Online Resource 2) showed a surface temperature of 5 °C, slowly decreasing to approximately 2 °C at 80 m depth. Lower salinity and density of the DB surface seawater compared to the deeper water was probably caused by ice melting and freshwater run-off from land. The surface SW temperature in the TRD seawater was 11.2 °C when sampled.

The concentrations of macronutrient and total organic carbon (TOC) in the Arctic (DB) and temperate (TRD) seawaters are shown in Online Resource 6. The macronutrient conditions differed between the DB and TRD surface seawater, with significantly lower concentrations of both N- and P-compounds in the former. Differences were also measured between the DB surface and subsurface seawater, with higher N and P concentrations in the subsurface than the surface seawater. However, significantly higher  $\text{NH}_4\text{-N}$  concentrations occurred in the TRD surface seawater (Online Resource 6).

### Oil droplets, temperature and oxygen

At the start of biodegradation experiment, the oil-droplet concentrations were between 3 and 4  $\text{mg L}^{-1}$  in the DB and TRD dispersions (average  $3.6 \pm 0.4 \text{ mg L}^{-1}$ ), while the median droplet sizes of the dispersions were  $17.1 \pm 2.3 \mu\text{m}$  (Fig. 1), close to the nominal conditions set on the oil-droplet generator. During the biodegradation experiment the droplet concentrations rapidly declined in all dispersions, and at the end of the experiment (64 days) the concentrations were reduced to an average of  $0.4 \pm 0.3 \text{ mg L}^{-1}$  in DB and TRD dispersions (11% of initial concentrations),



**Fig. 1** Oil-droplet concentrations (a) and median oil-droplet sizes (b) in dispersions with surface and subsurface seawater (SW) from DB and surface SW from TRD. Error bars represent standard deviations of triplicate analyses

irrespective of seawater source. The median oil-droplet sizes were rapidly reduced in the DB and TRD dispersions, averaging  $5.1 \pm 4.4 \mu\text{m}$  at the end of the experiment. In conjunction with the reduced oil-droplet concentrations, macroparticles expected to include oil and biomass were observed (Online Resource 7). The average seawater temperature in the DB and TRD dispersions during the experiment was  $5.4 \pm 0.9 \text{ }^\circ\text{C}$ . DO in the dispersions at the start of the study averaged  $9.0 \pm 0.5 \text{ mg L}^{-1}$ , and was never lower than  $5.9 \text{ mg L}^{-1}$  in any flask during the study, averaging  $7.6 \pm 1.2 \text{ mg L}^{-1}$  at the end of the study (day 64).

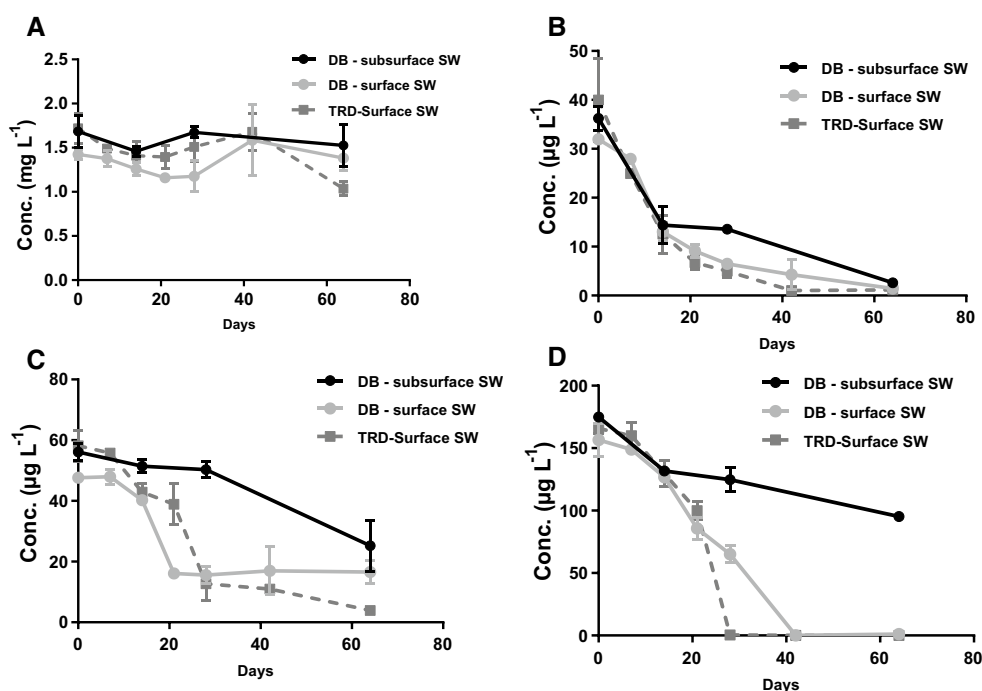
## Depletion and biotransformation of targeted oil compounds

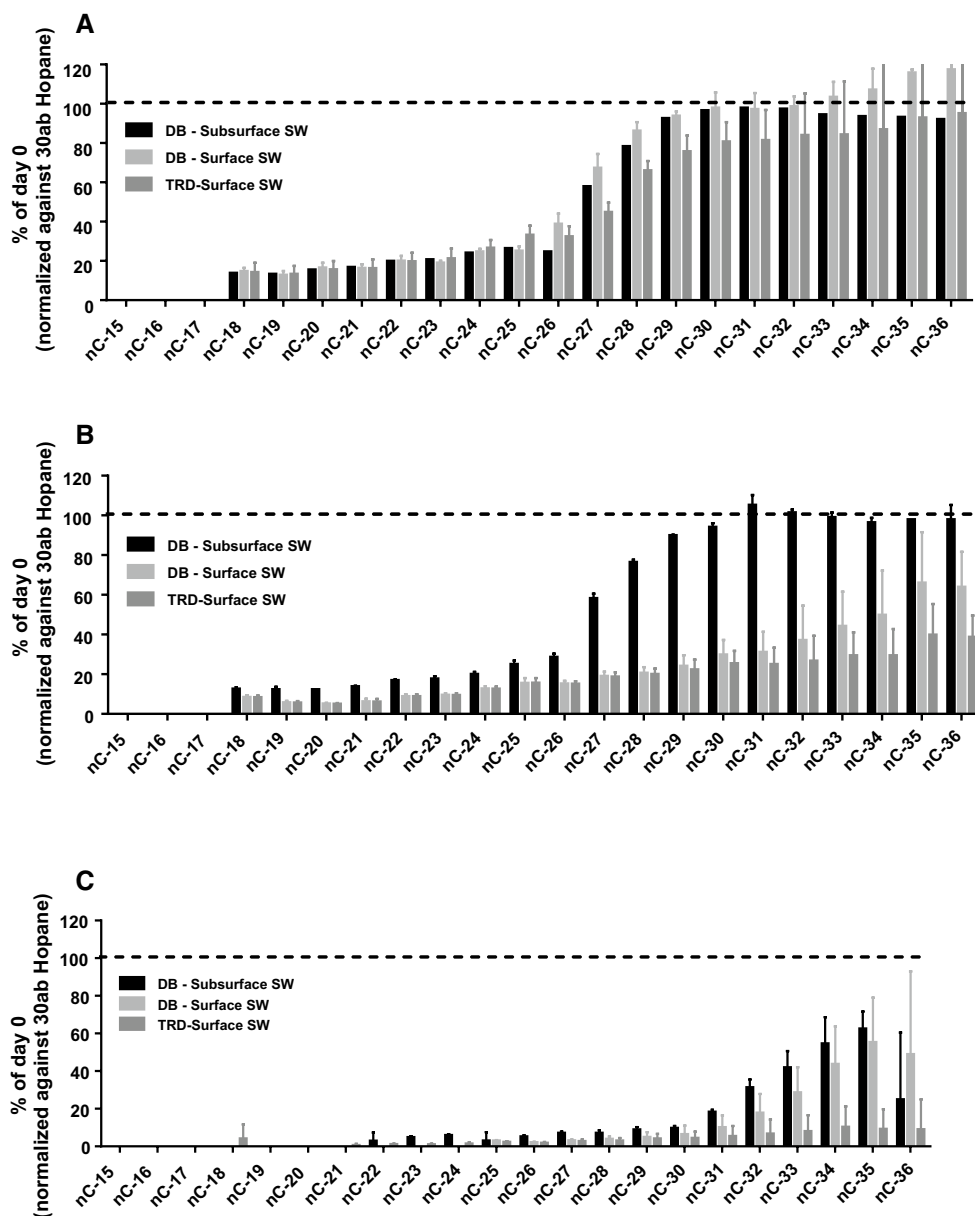
Measurements of TEM concentrations substantiated that reductions in oil-droplet concentrations were not the result of oil disappearances in the dispersions, since TEM concentrations were only negligible or moderately reduced compared to the oil-droplet concentrations. Only the dispersions in the TRD surface seawater showed significant reduction in TEM concentrations ( $p=0.0047$ ) during the experiment, with  $39.4 \pm 7.0\%$  decline after 64 days. The dispersions in DB surface and subsurface seawater showed insignificant depletion ( $p=0.6297$  in surface and  $0.5399$  in subsurface seawater), as shown in Fig. 2a. However, *n*-alkane depletion was substantial, with  $>90\%$  reduction in all dispersions (averaging  $95.4 \pm 2.3\%$ ) after 64 days of incubation (Fig. 2b). Depletion of 2- to 5-ring PAH was more than 50% in all dispersions after 64 days degradation period (Fig. 2c),

but higher in TRD surface seawater ( $93.3 \pm 0.2\%$ ) than in DB surface seawater ( $65.4 \pm 7.5\%$ ) and subsurface seawater ( $55.4 \pm 12.6\%$ ). Reductions of VOC compounds were faster in the TRD and DB surface seawater (average  $99.7 \pm 0.4\%$ ) than in the DB subsurface seawater ( $45.6 \pm 1.0\%$ ) after 64 days (Fig. 2d).

Biotransformation was examined in the dispersions after normalization against the persistent biomarker  $17\alpha(\text{H}),21\beta(\text{H})$ -hopane (30ab hopane), commonly used in biodegradation studies (Prince et al. 1994; Wang et al. 1998). No depletion of 30ab hopane was measured during the experiment (Online Resource 8). Biotransformation of single *n*-alkanes in all dispersions were insignificant after 7 days of incubation (not shown), while transformation of  $n\text{C}_{15}$ – $n\text{C}_{26}$  alkanes were determined in all dispersions after 14 days of incubation (Fig. 3a). Biotransformation was faster for  $n\text{C}_{15}$ – $n\text{C}_{26}$  alkanes than  $>n\text{C}_{26}$  alkanes in DB and TRD dispersions, and degradation of the larger alkanes occurred faster in the DB and TRD surface seawater than in the DB subsurface seawater (Fig. 3b, c). After the 64 days biodegradation period, the depletion of the higher *n*-alkanes ( $>n\text{C}_{30}$ ) was less complete in both DB seawaters compared to the TRD seawater (Fig. 3c), except for  $n\text{C}_{36}$ . However, a low concentration of this *n*-alkane resulted in a high standard deviation (Fig. 3c). The biotransformation of single 2- to 4-ring PAH compounds was also faster in the DB and TRD surface seawater than in the DB subsurface seawater, with more extensive depletion in the TRD than the DB seawater at the end of the experiment. The biotransformation followed a pattern

**Fig. 2** Concentrations of TEM (a),  $n\text{C}_{10}$ – $n\text{C}_{36}$  alkanes (b), total 2- to 5-ring PAH (c) and total VOC (d; the sum of  $\text{C}_5$ – $\text{C}_{10}$  alkanes and monoaromatic hydrocarbons) in seawater (SW) from DB and TRD. Error bars represent standard deviations of triplicate analyses





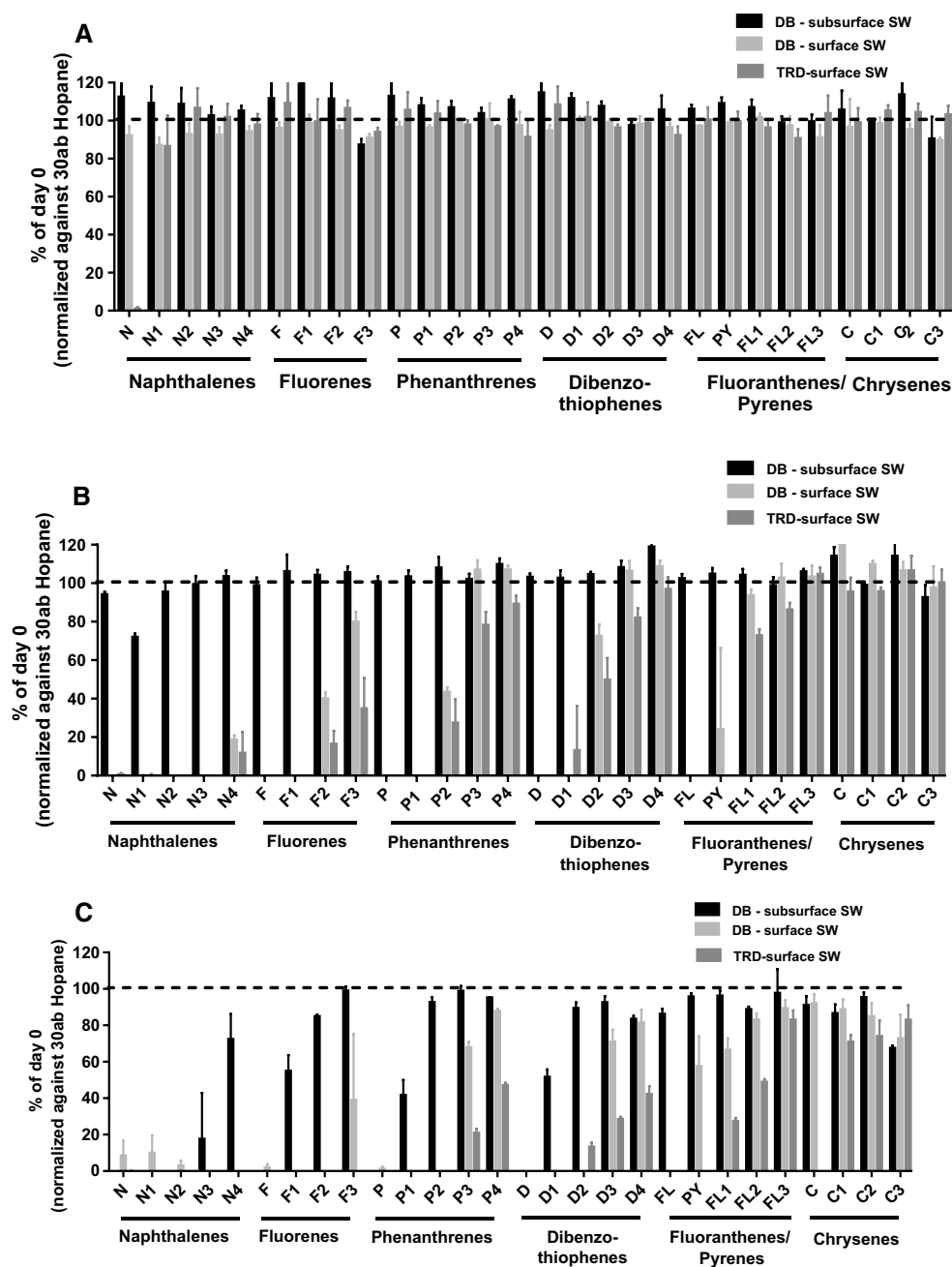
**Fig. 3** Biotransformation (% of day 0) of individual semivolatile  $n$ -alkanes ( $nC_{15}$ – $nC_{36}$ ) in oil dispersions with seawater (SW) from DB and TRD, after normalization of target analytes against 30ab hopane.

The data are shown after incubations for 14 days (a), 28 days (b) and 64 days (c). Error bars represent standard deviations of triplicate analyses

with slower transformation by increasing alkyl substitution (Fig. 4), in agreement with previous measurements of chemically dispersed oil (Brakstad et al. 2015a). Depletion of single volatile compounds (alkanes and BTEX) were also examined, although these could not be normalized against any biomarker. Typically, the BTEX were depleted slower in the DB subsurface seawater than in the DB and TRD surface seawater (Online Resource 9).

### Biotransformation rates and half-lives of oil compound groups

The SVOC and VOC compounds analysed by GC-FID, GC-MS and P&T GC-MSn, with boiling point ranges of 27 – > 500 °C, are considered to represent 70–80% of typical crude oils (Pasquini and Bueno 2007). These oil fractions were separated in 23 of total 25 OSCAR volatile and



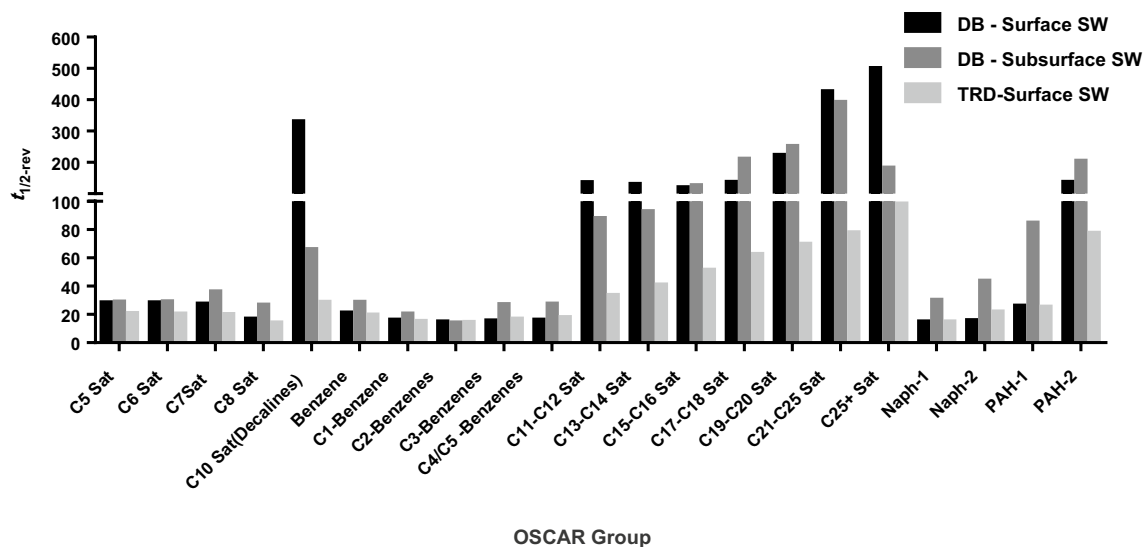
**Fig. 4** Biotransformation (% of day 0) of individual semivolatile 2- to 4-ring PAH in oil dispersions with seawater (SW) from DB and TRD, after normalization of target analytes against 30ab hopane. The data

are shown after incubations for 14 days (a), 28 days (b) and 64 days (c). Error bars represent standard deviations of triplicate analyses

semivolatile groups described in Online Resource 4 (Reed et al. 2000), but concentrations of phenols and  $C_9$  saturates in the Troll oil were below analytical LOD and could not be included in the calculations. Revised biotransformation half-lives ( $t_{1/2-rev}$ ; see Materials and methods and Online Resource 5) determined for each of these groups, including  $C_5$ – $C_{36}$  saturates, monoaromatic hydrocarbons, naphthalenes, and 2- to 5-ring PAH, are shown in Fig. 5, based

on the transformation rate data shown in Online Resource 10. Revised half-lives of the compound groups ranged from 15 to > 500 days, with the small aromatic hydrocarbons (BTEX and naphthalenes with the shortest half-lives (15–44 days). These compounds showed generally shorter biotransformation half-lives in the DB and TRD surface seawater (median half-lives of  $15.7 \pm 2.2$  days in DB and  $16.9 \pm 2.7$  days in TRD) than in the DB subsurface





**Fig. 5** Revised half-lives ( $t_{1/2-rev}$ ) of VOC and SVOC oil compound groups determined from oil biotransformation kinetics in DB surface and subsurface seawater (SW) and TRD surface SW. The revised half-lives were determined as described in Materials and methods

seawater (median half-life  $27.4 \pm 9.1$  days). The revised half-lives of the semivolatile saturates ( $C_{10}$ – $C_{25}$  + Sat) were considerably shorter in the TRD surface seawater (median half-lives of  $57.0 \pm 23.8$  days) than in the DB seawaters (median half-lives of  $180 \pm 120$  days in surface and  $154 \pm 109$  days in subsurface seawater). The slower biotransformation of the quantitatively predominant saturates in the DB surface seawater could possibly be related to the low macronutrient concentrations in this water (Online Resource 6). Finally, the half-lives of the larger 4- to 5-ring PAH (PAH-2) were also higher in the DB than the TRD seawater. Comparison of the data sets showed that the TRD data differed significantly ( $t$  test,  $p \geq 0.0005$ ) from both DB data sets, while the DB data did not differ significantly from each other ( $p = 0.3554$ ). The revised half-lives of each seawater source (Online Resource 10) were also compared to half-lives determined from rate coefficients, in which lag-periods were not included in any calculations (Online Resource 11). No significant differences were determined ( $t$  test,  $p$  value ranging from 0.0905 to 0.3472) between calculation methods, although longer half-lives were determined for several saturates when lag-periods were not included in the calculations (Online Resource 11).

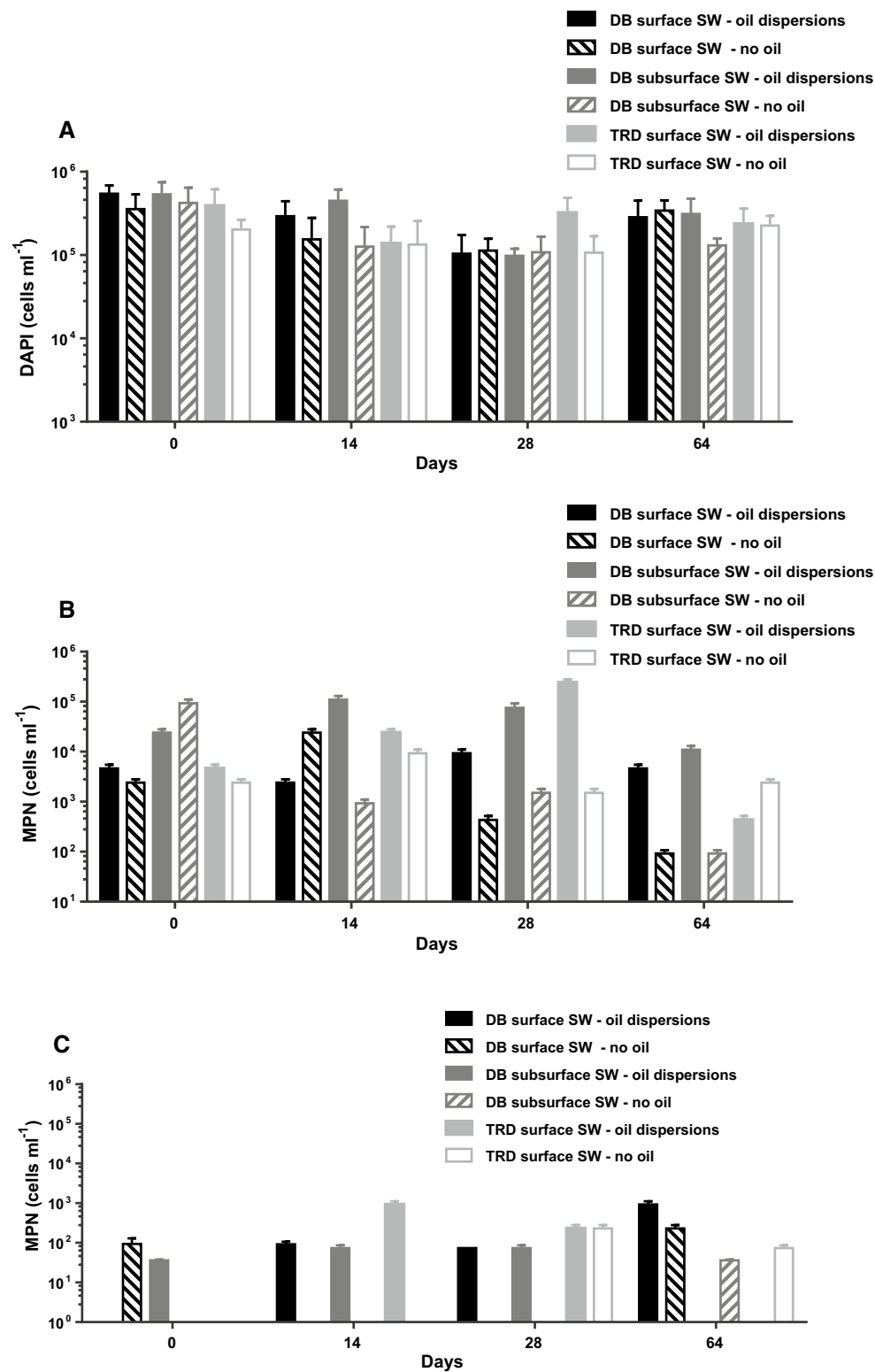
In the OSCAR model, temperature calibrations of biodegradation rates are performed by  $Q_{10}$  calculations, using biotransformation data provided from experiments at  $13$  °C. Comparison of rate coefficients and half-lives calculated by  $Q_{10}$  approach ( $Q_{10}=2$ ) for  $5$  °C from experimental data at  $13$  °C (Brakstad et al. 2018a) showed that rate coefficients of semivolatile OSCAR groups were overestimated by the  $Q_{10}$  method (Online Resource 12) when compared to the

experimental data at  $5$  °C (Online Resource 11), and all experimental data sets differed significantly from the  $Q_{10}$  calculations ( $t$  test,  $p \leq 0.001$ ).

### Microbial concentrations and communities

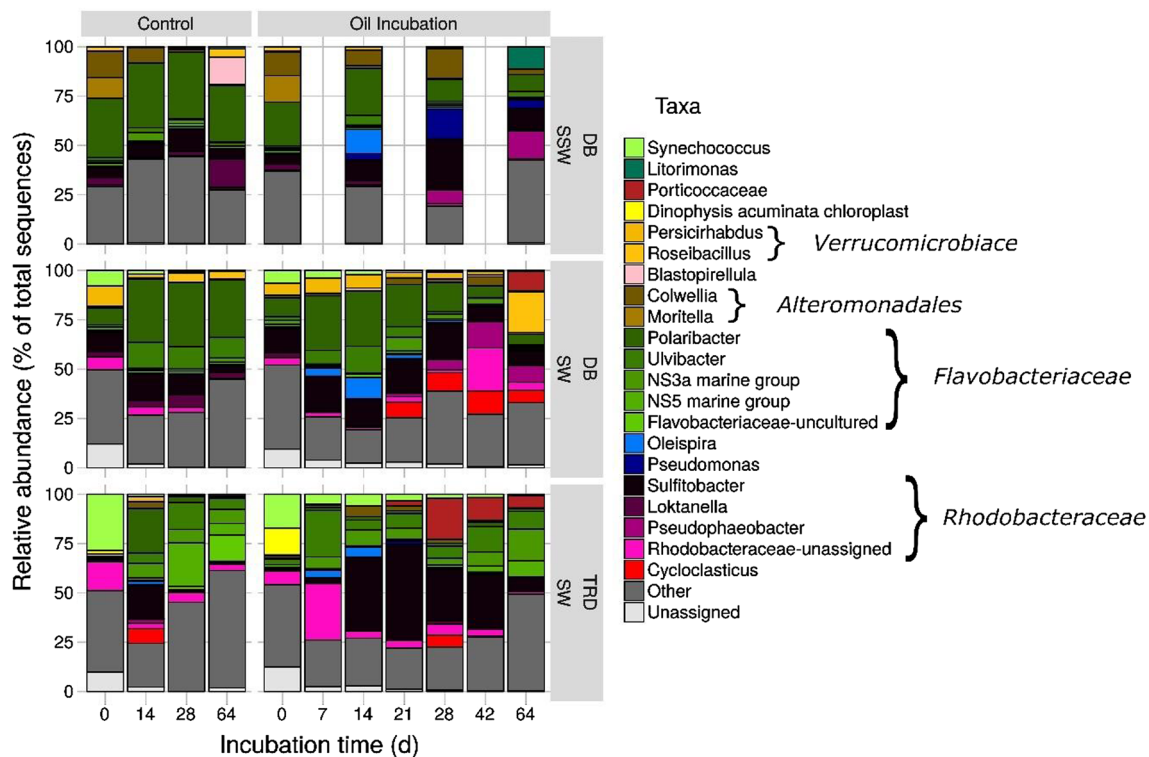
The low oil concentrations used in the current study did not result in increased total concentrations of microbes in the dispersions, although the concentrations were generally higher in the oil dispersions than in seawater without oil, which was incubated at the same conditions (Fig. 6a). MPN analyses of HP (Fig. 6b) showed in general stimulation of growth in oil dispersions, compared to in seawater without oil after 28 and 64 days of incubation. The concentrations of ODP (Fig. 6c) were low, but they were mainly higher in the TRD seawater than DB seawater after 14 and 28 days, which represented the main period of  $n$ -alkane, PAH and VOC compound degradation (see Fig. 2). However, ODP were also detected in several samples without oil, particularly at the end of the biodegradation period (day 64).

The microbial communities at the start of the biodegradation experiment differed between the seawater sources. The DB subsurface and surface seawater was predominated by *Polaribacter* (Flavobacteriaceae) and *Sulfitobacter* (Rhodobacteraceae), with additional abundances of *Colwellia* and *Moratella* (Alteromonadales) in DB subsurface seawater and *Persicirhabdus* (Verrucomicrobiaceae) in DB surface seawater. These bacterial groups were also abundant in the original DB seawaters sampled and filtered before transport (results not shown). TRD communities were predominated by Rhodobacteraceae and *Synechococcus* (Fig. 7). Bacterial successions were observed in the oil dispersions,



**Fig. 6** Microbial concentrations determined as total concentrations by epifluorescence microscopy (**a**), and MPN enumeration of heterotrophic prokaryotes (**b**) oil-degrading prokaryotes (**c**) in seawater

(SW) from DB and TRD. Error bars represent standard deviations of epifluorescence counts, or 95 % confidence intervals of MPN enumerations



**Fig. 7** The relative abundances among the microbial taxa (genus level) that represented  $\geq 10\%$  of the total sequences in each sample. The relative abundances are shown by sampling day for seawaters

with changes after 14–28 days of incubations. The abundances of *Oleispira* (Oceanospirillaceae) and *Ulvibacter* (Flavobacteriaceae) increased in all oil dispersions during the first 14 days, while *Polaribacter* (Flavobacteriaceae) became increasingly abundant only in the DB surface seawater. In the same period *Sulfitobacter* (Rhodobacteraceae) became increasingly abundant in the DB subsurface and TRD surface seawaters. Later in the biodegradation period (28–64 days) taxa with increased abundances in the oil dispersions included the genera of *Pseudophaeobacter* (Rhodobacteraceae) in both DB surface and subsurface seawater, *Litorimonas* (Hyphomonadaceae) and *Pseudomonas* in DB subsurface seawater, while *Cycloclasticus* (Piscirickettsiaceae) and *Porticoccaceae* abundances increased in both surface seawaters from DB and TRD.

## Discussions

The seawater sampled from DB showed higher N/P ratios in the surface than the subsurface seawater. However, nitrate concentrations were higher in the subsurface seawater. The seawater was collected in September and could be affected by the previous algal blooming season, resulting in limited nitrate concentrations in the surface water. While the DB

without oil (Control) and in oil dispersions (Oil Incubation) with sub-surface seawater from DB (DB SSW), surface seawater from DB (DB SW) and TRD surface seawater (TRD SW)

macronutrient conditions show highly seasonal variations, the deeper water in DB is more stable (Hansen et al. 2012). Biological activities in the DB surface seawater were further substantiated by higher ammonium concentrations than in the subsurface DB seawater. The high ammonium concentrations occurring in the TRD surface seawater indicated both higher N-input and biological activities in this seawater compared to the DB surface seawater. Previous measurements of macronutrients in TRD subsurface seawater (80 m depth) corresponded more to the DB subsurface seawater, with low ammonium concentrations (Brakstad et al. 2015a, b).

In the current studies, the oil was premixed with a dispersant, and dispersions were generated with oil-droplet sizes relevant for effective dispersant treatment of oil spills ( $17.1 \pm 2.3 \mu\text{m}$ ), and low oil-droplet concentrations were used (close to  $3 \text{ mg L}^{-1}$ ). It has been suggested that the use of dispersants may suppress the activities of oil-degrading bacteria, altering the microbial community composition through selection for potential dispersant-degrading bacteria like *Colwellia* in favour of oil-degrading bacteria (Kleindienst et al. 2015). No such suppression was observed in our studies. Recent studies with physically dispersed oil of similar oil-droplet sizes, premixed or not premixed with dispersant, have also shown that microbial communities

and hydrocarbon biodegradation were comparable in the oil dispersions with and without dispersant premixing. The studies also showed that in solutions of dispersant alone (no oil), microbial communities were comparable to those in seawater without oil and dispersant (Brakstad et al. 2018b). The oil rather than the dispersant was therefore the driver for the microbial successions and biodegradation when dispersions were used.

During the biodegradation experiment, oil-droplet concentrations were rapidly reduced, probably as a combined result of droplet coalescence, glass wall attachment, and the observed flocculation of oil and biomass into macroparticles. The reduction in oil-droplet concentration has been measured in previous studies with dispersed oil biodegraded in subsurface (80 m depth) seawater from TRD (Brakstad et al. 2015a). The flocculation process and the characterization of these particles, consisting of the original oil, degradation products and biomass, has been reported in several studies (Macnaughton et al. 2003; Hazen et al. 2010; Bælum et al. 2012). Oil concentrations, measured as TEM, declined only moderately (TRD seawater) or negligible (both surface and subsurface DB seawater) in these studies, and the reductions were slower than previously measured in cold TRD subsurface seawater (4–5 °C) with a paraffinic Macondo oil (Brakstad et al. 2015a). The oil properties may have influenced the TEM degradation, since the Troll oil used in the current studies was an UCM-rich oil with low concentrations of rapidly degradable *n*-alkanes. This was further substantiated by the fact that target-specific degradation of *n*-alkanes here was comparable to the studies with the Macondo oil (Brakstad et al. 2015a). The *n*-alkanes were faster biotransformed than PAH and VOC, in particular in the DB surface seawater. Several studies have shown that *n*-alkanes from dispersed crude oils may degrade faster than many PAHs in seawater at low temperature (McFarlin et al. 2014a, b; Ribicic et al. 2018; Brakstad et al. 2018b), in agreement with the assumption that hydrocarbon degradation rates follow the order *n*-alkanes > branched alkanes > low molecular weight aromatics > cyclic alkanes (Perry 1984).

In all seawaters, single target analyte analyses showed slower *n*-alkane biotransformation with increasing chain-length, while 2- to 4-ring PAH transformation was reduced by increasing alkyl substitution, in agreement with previous measurement (Venosa and Holder 2007; Prince et al. 2013; McFarlin et al. 2014a, b; Brakstad et al. 2015a). However, both *n*-alkane and 2- to 4-ring PAHs were degraded slower in the DB subsurface seawater than in the two other seawater sources. Recent oil biodegradation studies with subsurface seawater from DB (150 m depth), using crude oils from Oseberg Blend (100 mg L<sup>-1</sup>; 5 °C incubation temperature) or Draugen (2.5–10 mg L<sup>-1</sup>; 2 °C incubation temperature), showed that *n*-alkane biotransformation was comparable to our results. However, only C<sub>0</sub>- and C<sub>1</sub>-naphthalenes

showed evidence of biodegradation after 71 days of incubations, while fluorenes, dibenzothiophenes, phenanthrenes, chrysenes or pyrenes did not degrade at any measurable extent in these studies (Kristensen et al. 2015; Scheibye et al. 2017). Although PAH biotransformation in DB subsurface seawater was slow in our studies, even slower PAH transformation was therefore measured with the water from 150 m depth. One possible explanation for the obviously decreased PAH biotransformation by increasing water depth could be that the more stable subsurface seawater (Hansen et al. 2012) represented a more pristine environment with no previous exposure to hydrocarbon contamination. However, both, oil quality, concentration and experimental conditions probably affected the differences in the results. In agreement with our results, other biodegradation studies of oils and hydrocarbons with Arctic seawater have shown the potential for biodegradation of PAH compounds (Bagi et al. 2014; McFarlin et al. 2014b; Ribicic et al. 2018).

Biotransformation half-lives as determined for the OSCAR groups, showed differences between the oil dispersions with respect to compound group water solubilities. While water-soluble groups like C<sub>5</sub>- to C<sub>8</sub>-saturates, benzenes and naphthalenes showed mainly comparable results between the water sources, the half-lives of the poorly soluble saturates were longer in the DB than the TRD seawaters. It must be emphasized that each oil compound group used in the OSCAR model represents individual compounds with different degradation rates, as exemplified with the different half-lives between non-alkylated and alkylated PAHs, and between *n*-alkanes and other unresolved compounds in the saturate groups, in this and other studies (Brakstad et al. 2015a; Brakstad et al. 2018a; Ribicic et al. 2018). The degradation rates will therefore be dependent largely on the quantitatively predominant compounds of each group. In a biodegradation study of a paraffinic oil (Macondo) with two oil-droplet sizes (median droplet diameters of 10 μm and 30 μm), using TRD subsurface seawater and 5 °C incubation temperature, revised half-lives of saturate and aromatic OSCAR oil compound groups ranged between 3 and 72 days, and 4–128 days, in the 10 and 30 μm dispersions, respectively (Brakstad et al. 2015a). The Macondo oil half-lives in the TRD subsurface seawater were mainly comparable to the Troll oil half-lives in the TRD surface seawater for the quantitatively predominant saturates (C<sub>19</sub>–C<sub>25</sub> + Sat) and 3-ring PAH (PAH-1), while biotransformation rates were faster in both Macondo oil-droplet size dispersions for volatile aromatics (benzenes), smaller saturates (C<sub>10</sub>–C<sub>18</sub> Sat), naphthalenes (Naph-1 and Naph-2) and 4- to 5-ring PAH (PAH-1) (Brakstad et al. 2015a). The revised half-lives thus differed generally more between the two oils for the aromatic than the saturate groups, and these differences may be the result of different oil properties, since the Macondo oil is an highly dispersible paraffinic oil (Brakstad et al. 2014), while

the Troll oil is a naphthenic oil. Although a recent study showed comparable biotransformation half-lives between oils with different properties when incubated in natural seawater at 13 °C (Brakstad et al. 2018a), oil compound biotransformation differences may increase with reduced temperature, partly because of differences in oil properties like viscosity, pour point and wax precipitation (Margesin and Schinner 1999). Spatial and temporal differences in seawater properties may also have influenced the different results in the TRD surface and subsurface seawater, for instance ammonium concentrations differed between the TRD seawater samples (Online Resource 6).

The comparison of experimental data and  $Q_{10}$  calculations indicated that the  $Q_{10}$  method overestimated biodegradation of semivolatiles OSCAR groups at 5 °C when compared to experimental data, even when compared to the data from the same seawater source (Brakstad et al. 2018a). Bagi et al. (2013) performed  $Q_{10}$  calculations for several oil biodegradation data sets and observed high variations in values. They concluded that, considering non-nutrient limitations (using low oil concentrations), differences in bacterial community compositions may be a key parameter (Bagi et al. 2013), but also the oil properties may become important parameters at low temperatures (Margesin and Schinner 1999).

Examination of microbial communities in the seawater sources were partly in agreement with results from previous analyses of seawater from the Western Greenland coast, with abundances of Rhodobacteraceae, Colwelliaceae, Oceanospirillaceae (Frette et al. 2010; Dziallas et al. 2013). Also the TRD communities were in agreement with recent data from TRD surface seawater was predominated by Rhodobacteraceae, *Synechococcus* and non-identified groups, in agreement with data from previous studies (Brakstad et al. 2015b). Although the TRD seawater was collected at a temperature of 11 °C, we have previously detected typical psychrophilic oil-degrading bacteria in this water (Lofthus et al. 2018; Ribicic et al. 2018).

Several of the community members emerging in the dispersions during the biodegradation period are associated with oil biodegradation. Oceanospirillaceae is associated with alkane metabolism (Hazen et al. 2010; Redmond and Valentine 2012; Dubinsky et al. 2013; Brakstad et al. 2015b; Wang et al. 2016), in agreement with the abundances early during the oil biodegradation period. This family was represented by the genus *Oleispira*, known to biodegrade alkanes at low seawater temperatures (Yakimov et al. 2003; Coulon et al. 2007; Golyshin et al. 2010; Lofthus et al. 2018). *Sulfitobacter* and *Polaribacter* are also associated with oil biodegradation in seawater or with oil-contaminated marine environments (Deppe et al. 2005; Brakstad et al. 2008, 2018b; Guibert et al. 2012; Dubinsky et al. 2013; Ribicic et al. 2018). The changes of the microbial successions during

the biodegradation period were associated with increasing abundance of bacteria able to metabolize aromatic hydrocarbons, as shown by the emergences of *Cycloclasticus* and Porticocccaeae. *Cycloclasticus* is able to transform a variety of monoaromatic hydrocarbons and PAHs in the marine environment (Geiselbrecht et al. 1998; Kasai et al. 2003; Wang et al. 2008), while members of the gammaproteobacterial family Porticocccaeae have been involved in biodegradation of PAH from oil, (Gutierrez et al. 2012). Interestingly, high abundances of *Colwellia* were observed in the DB subsurface seawater, a group of bacteria associated with biodegradation of a variety of hydrocarbons at low seawater temperatures (Mason et al. 2012; Redmond and Valentine 2012; Valentine et al. 2012).

## Conclusions

The results from these studies showed that seawater from an Arctic environment in Western Greenland contained microbial communities with the capacity to biodegrade oil compounds in dispersed oil. When compared to a surface seawater from a temperate Norwegian fjord, using similar biodegradation temperatures, *n*-alkanes and 2- to 4-ring PAH in a dispersed naphthenic crude oil were degraded slower in the Greenland seawater, particularly in a subsurface seawater. When biodegradation rates and half-lives of 21 oil compound groups representing 70–80% of the oil were compared, both surface and subsurface Greenland seawater biodegraded the oil compound groups significantly slower than the temperate seawater at the same temperature. However, degradation of volatile and semivolatiles aromatic oil compounds associated with acute effects (Faksness et al. 2015), differed less than the quantitatively predominant saturates. The Greenland and Norwegian seawater differed with respect the abundances of microbial communities, and oil compound biodegradation was associated with different bacterial groups in the seawater from the different environments. However, typical oil-degrading bacteria were present in all the seawater sources and increased in abundances during oil biodegradation. This and other studies show that hydrocarbons are biodegraded in Arctic seawater after an oil spill, but degradation rates at low temperatures may vary in seawater from different environments. These data will therefore be of importance when predicting the environmental fate and potential impacts of oil spills in the Arctic. Numerical oil spill prediction tools, like the OSCAR model, which include biodegradation as one of the processes for predicting the fate of spilled oil, may use biodegradation data from a reference temperature adjusted to the ambient temperature by a  $Q_{10}$  approach. From the results of this study, it should be considered using experimental data for the relevant Arctic environment directly if available, rather

than to temperature-calibrate data from temperate seawater environments.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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